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HOW TO COLLECT AND PREPARE FOREST INSECTS, DISEASE ORGANISMS, AND PLANT SPECIMENS FOR IDENTIFICATION

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By

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I. Introduction

Foresters, technicians, and pest control specialists often find it necessary to ship insects and disease specimens for identification. Almost invariably, poorly prepared or badly packaged specimen arrive damaged--often to the point that identification is impossible. Proper specimen preparation reflects well on the consideration and professionalism of the sender, and such shipments are more likely to receive prompt and careful attention. This guide is prepared as an aid in collecting, preparing, and shipping specimens for identification.

Specimen preservation, packaging and shipping is simple but requires care. Techniques vary between different kinds of insects and disease samples. Included here are some basic recommendations which are applicable to most types of specimens or techniques which can be generally applied to them all. For a more thorough discussion, consult the list of suggested readings.

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II. Plant Specimen Collection

If a tree appears to be unhealthy, examine it and its environment closely. Examine other trees of the same species in the vicinity as well as those of other species. Determine, if possible, whether other similarly affected trees occur in groups or are scattered more or less randomly through the stand.

Next, check the immediate surroundings of affected trees. Look for disturbances of soil and other vegetation and evidence of underground systems such as utility lines. Observe a wide area and adjacent farmland, if any, for evidence of herbicides or other pesticide use. These environmental factors are especially important in shade tree disease diagnosis. Be as specific as possible about the presence of streets, buildings, and cultural practices such as pruning.

Look at the symptoms. Has the tree suddenly turned brown or has it defoliated gradually while remaining green? Has the tree begun to decline from the top down? Has the bark loosened? Did the tree wilt while still green? Are the leaves curled? All of these and any other similar "expressions" by the tree are called symptoms and are the first consideration in diagnosis and possible specimen selection. While studying them, think back to conditions over the past year. Were there extremes in temperature, droughts of any consequence or other factors detrimental to plant growth and development?

While examining the tree for symptoms, look also for signs. Signs are physical evidence of causes, i.e., insects and disease organisms. Fruiting

bodies, such as conks and mushrooms, are the signs produced by fungi. They may be found growing on the host tree itself, on the ground around it or on nearby stumps and logs. Examples of insect signs include frass and cast larval skins, and the insects themselves. Carefully collect and prepare suspicious specimens for shipment (see Section IV). Not all fungi and insects found in the vicinity of the unhealthy tree may be causal. Try to establish some correlation between the affected tree itself, its unhealthy or dead neighbors and the specimen(s) you collect.

Smaller trees are easier to examine than large ones and may permit a more thorough field diagnosis. No matter how large or small, however, the entire tree should be examined, if possible. When present, foliage can be an invaluable aid. However, it may only be symptomatic of a problem centered elsewhere. For example, wilted leaves or top dieback do not necessarily indicate the presence of foliar insects or diseases but may instead result from the effects of a stem or root pest. Check the host for loose or dead bark, for cankers on limbs and stems, insects or insect parts, and for root and root collar damage. However, do not submit bark by itself unless cankers, fruiting bodies, or possibly characteristic insect damage is present on it. Many organisms inhabit the bark and thus isolation from this material produces much contamination.

If possible, insects to be collected should be killed in the field. Soft bodied specimens such as aphids and beetle larvae may simply be dropped in alcohol. Moths and most hard bodied insects should be killed in a killing jar. The best killing jars are wide-mouthed, with about 3/4" of hardened

plaster of paris in the jar bottom. A few drops of ethyl acetate (nail polish remover) are added to the plaster of paris where they are quickly absorbed. Some tissue added to the killing jar will help prevent moths from damaging their wings against the jar sides and bottom.

III. Insect Specimen Preparation

A. Adult Insect Preparation

1. Relaxing

If insects lose flexibility before pinning, rigormortis develops, often resulting in a body distortion.[2/] In such cases, use a "relaxing chamber" to make them flexible. Relaxing chambers are very simple and inexpensive to construct. One type is simply an airtight covered dish which contains moist paper towels or damp sand (Figure 1). A small amount of fungus inhibitor (such as Lysol®) should be added.

When insects are removed after the relaxation period (36-48 hours), they should be pliable. At this time, their appendages can be more attractively or practically positioned--usually with temporarily placed insect pins. Rigormortis again sets in, but this time the insects are "frozen" so that their important identification characters are more discernible.

[2/] Shipment of live insects is beyond the scope of this publication (see Section VII-A).

2. Pinning

In most (but not all) cases, adult insects should be pinned with pins specifically designated as "insect pins". Pin placement for different kinds of insects varies with their type. Large hard-bodied insects, such as most long-horned wood boring beetles, are pinned directly through the body. Pin positioning varies slightly with the classification of the insects (beetles, flies, moths, true bugs, etc.). Figure 2 shows proper pinning areas for more common types of larger insects.

Small insects, such as most bark beetles, cannot accommodate relatively large pins, therefore, they must be placed on "points", which in turn are pinned. Points are triangular, tiny pieces of rigid paper. They can be made with scissors, or more easily with a "point maker". Point makers resemble hole punches and quickly punch out uniform-sized points (Figure 3). To position bark beetle sized insects on a point, the small end of the point is bent down and a tiny drop of glue is applied to the deflection. Then the insect is attached at its right side (Figure 4). Do not use too much glue as this may obscure the middle and opposite side of the insect. Ordinary glue is acceptable but a clear acetate cellulose cement (such as ambroid) is preferable since it does not become too brittle. Ambroid is available at variety stores in small quantities.

Insects which are still smaller, such as wasps and flies, are mounted on unbent points. Minute insects (such as mosquito-sized

parasitic wasps and tiny moths) are mounted on minute (tiny) pins which are inserted in cork which is pinned with a regular insect pin.

Insects on pins or points should be placed at a height of one inch. This height can easily be determined through use of a "pinning block" (Figure 5). Pinning blocks can be homemade or purchased from biological supply houses. Specimen height is simply equal to the maximum depth reached by the pin in the deepest of three holes. The function of holes 2 and 3 will be discussed under "labeling".

Because of their structure, moths require special treatment. While still pliable, or after relaxation, moths must be pinned directly through the middle of the thorax and positioned on a "spreading board" (Figure 6). Paper strips are then pinned over each set of wings parallel to the torso. A needle is then used to gently spread the wings so that they are fully extended. If a spreading board is not available, this same basic procedure can be used on a broad, flat surface, but the insect must be positioned upside down to avoid leg damage. When the abdomen feels firm to the touch, the specimen has sufficiently dried and can be labeled.

As mentioned earlier, not all adult insects should be pinned. Soft-bodied specimens (such as aphids and scales) must be preserved in alcohol.

B. Immature Insect Preparation

Most (but not all) immature insects should be preserved in alcohol. Make every effort to use ethyl alcohol. If ethyl is unavailable, isopropyl may be used for temporary preservation. Liquid preservation is obviously practical for such insects as moth and sawfly larvae. Here too, forethought will help insure proper preservation. There are several liquid formulae used to preserve specimens. A common, simple, and functional mixture is 95 parts of 70-80 percent ethyl alcohol with five parts glycerine. Glycerine is easily obtainable from drugstores. Insects so preserved are usually kept in vials with solid rubber stoppers.

If possible, insects to be preserved in vials should be killed by dipping in boiling water. This helps insure better color retention. Immediately after death, the insect is placed in a full vial of the alcohol-glycerine mixture. A specimen label should accompany the insect (labeling will be discussed in detail later). With an insect pin placed against the interior of the vial, the rubber stopper is forced deep into the vial as the pin is simultaneously withdrawn. This pulls surplus air out of the vial as a stream of tiny bubbles, thus helping to insure an airtight fit (Figure 7).

Because the body contents of a large specimen may dilute the preservative, it is best to replace the original alcohol with fresh alcohol after a day or two.

As mentioned earlier, not all immatures should be preserved in alcohol. Hard-bodied nymphs for example (e.g., conebugs), should be pinned as adults.

C. Insect Labeling

Proper labeling is an integral part of specimen submission. The insect identifier can make use of the collection date and location to help narrow down or confirm identification. Figure 8 is an enlarged example of a typical label. Line 1 shows the collection place. Lines 2 and 3 are for the collection date and collector's name, respectively. If the insect is pinned through its body, the location label is pinned through the center and pushed to level 2 (or 3/4") of the pinning block. With specimens on points, move the pin toward the beginning of the label to provide better "balance" of the insect and label. Level 3 of the pinning block is for the specimen identification label. It is not used by the submitters unless they request confirmation of an identification. Additional information such as host data or specimen number may also be placed on label 3. With labeling too, proper care insures functional, long-lasting data. Letters should be printed in block style. Labels should be very small but neat. They may be printed in either India ink or pencil (preferably India ink). Because the lettering is so small, many workers prefer to use rapidograph pens. Fountain pens are impractical and fiber points are unacceptable. Labels for alcohol-preserved specimens are simply included loosely in the vial. The alcohol will not affect quality ink or lead penciling.

D. Insect Packaging

Poor specimen packaging is a never-ending source of frustration for those assigned responsibility for insect identification. When packaging insect specimens, the shipper should plan for the parcel to be badly abused in the mails. Provision for rough handling should be made first within the specimen area itself. With pinned specimens, the pinning base should be tight, thick styrofoam or a similar material. The base should fit tight in a box bottom. It should also be glued with a strong nonvolatile adhesive which will not corrode the styrofoam or box. Pins should be pressed deep into the styrofoam, but not to the point hwere they protrude or begin to protrude through the box bottom. If specimens are large, they should be accompanied by stabilizing pins which prevent them from turning in transit (Figure 9). The inside box edges should be lined with cotton which has been securely glued or pinned. Its purpose is to catch any insect appendages which may become dislodged in transit, thus preventing further damage. The lid should fit firmly on the box. Ideally, it too is fitted with a styrofoam inner top which fits securely over the knobs of the pins. The exterior should show instructions on how to remove the lid (e.g., "lift straight up" or "hinged here" followed by arrows). Under no circumstances should pinned and bottled specimens be sent in the same container. Vials should be wrapped in cloth or paper towels to prevent their contacting one another.

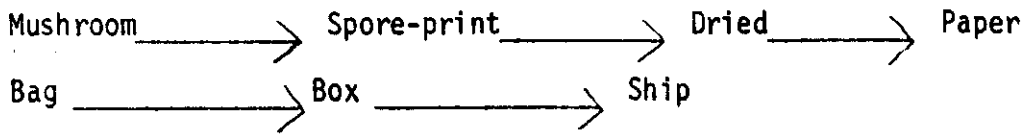
IV. Fungal Fruiting Body Collection and Preservation

In order for a disease specialist to identify a fruiting body correctly, the collector must follow certain steps. When submitting the fleshy, mushroom type, collect fresh mature and immature fruiting bodies, typical of those observed. At least six representative samples should be collected. While fresh, a spore-print should be made. This can be done by cutting the mushroom cap from the stalk and placing it with gills or pores down on a clean piece of paper that is half white and half black and then cover (Figure 10). Some experimentation may be necessary to determine when the print is definitive. Set up several caps and remove them after varying time periods and allow to air dry, fold papers so print does not rub off. Do not dry over heat.

Fleshy fungi shrivel and shrink upon drying--so much so, they will not resemble their fresh condition. Before drying, make note of basic shape, size and color of all parts (e.g., cap, stalk, gills) and include other information about the specimen and host.

Perennial conks are usually woody and require no particular handling for preservation. Fleshy fungi are quite another matter and must be given special care. Rapid drying until brittle in a drying chamber is necessary immediately after collection. Quick drying prevents other fungi, bacteria, and insects from destroying the specimen. The chamber can be made by placing a screen over two 100 watt bulbs. The screen and bulbs should be in a fire resistant box with an open top (Figure 11).

Dried fungal material should be placed in a paper bag for storage shipment. Plastic bags will not permit residual moisture to escape. This moisture may permit the growth of destructive bacteria and saprophytic fungi. The following flow chart summarizes the procedure for preparation and shipment of fresh fruiting bodies:



V. Collection and Preservation of Plant Material and Soil

When possible, collect whole plants along with some of the soil from around the roots. If the plant is too large for collection, examine and collect samples from different parts. In addition, collect samples from other plants showing different stages of the problem and from at least one that is healthy.

When collecting a branch or stem sample, take a wedge or section that includes both dead and apparently healthy tissue. This advancing margin frequently contains the living pathogen.

All plant materials should be kept moist and cool. Place host material in a plastic bag with moist paper toweling and put in a cool place. Vehicles parked in the sun in the summer can become hot enough to kill fungal pathogens. As a result, they cannot be isolated from the host.

Soil should also be placed in a plastic bag, but separate from the associated plant specimen. The soil should also be kept cool prior to shipment.

VI. Shipping of Insect and Disease Specimens

All material (including insects, fungi, plant material and soil) should be shipped in a sturdy carton or mailing tube. Cartons are definitely preferable for insect specimens. Pad the interior container with 2-3 inches of packing material surrounded by another box. Make sure it is protected from moisture. Include in the specimen box a description of the contents, host and host environment, your address and that of the recipient. Forest Service form 3400-1 (Figure 10) is one convenient checklist that can be used. Seal the container with cord or fiber tape. Place the words "CONTENTS: PLANT MATERIAL (or DEAD INSECTS) OF NO COMMERCIAL VALUE" on the package. Boxes with perishable specimens should be placed in a cool place until delivery to the carrier. If living plants or pests are among the contents, obtain a permit from the Animal and Plant Health Inspection Service (APHIS) and attach to the package.

With perishable specimens or a large number of preserved insects, contact the receiver to arrange for a convenient shipping date. Then, ship by the fastest means possible and early in the week to insure the specimens are not held by the carrier over the weekend.

VII. Miscellaneous Information

A. Shipment of Live Pests

Packaging and shipment of live insects is beyond the scope of this publication. The reader is referred to Entomological Techniques:

How to Work with Insects by Alvah Peterson for ideas. It should also be noted that shipment of live insect pests is subject to regulations issued by the U. S. Department of Agriculture Animal and Plant Health Inspection Service.

B. Acquiring Supplies

There are numerous biological supply houses throughout the United States which supply equipment needed for preserving and shipping insects. An Introduction to the Study of Insects by Borror, DeLong and Triplehorn contains a list. Local high school biology departments, extension agents, and colleges also can suggest suppliers. Ordinarily, no special equipment is necessary for disease specimen preparation.

C. Where to Send Specimens

Specimens collected by Forest Service foresters should be submitted to entomologists or pathologists with Forest Insect and Disease Management (State and Private Forestry). Homeowners and state agency foresters should check with their State Forester regarding specimen submission.

VIII. Suggested Readings

Borror, Donald J., Dwight M. DeLong, and Charles A. Triplehorn. 1964. An introduction to the study of insects. Holt, Rinehart and Winston, New York.

Hawksworth, D. L. 1974. Mycologists Handbook. Commonwealth Mycological Institute, Kew. 231p.

Knutson, Lloyd. 1976. Preparation of specimens submitted for identification to the Systematic Entomology Laboratory, USDA Bull. Entomol. Soc. Amer. 22:130.

Miller, Orson K. 1977. Mushrooms of North America. E. P. Dutan Company, New York.

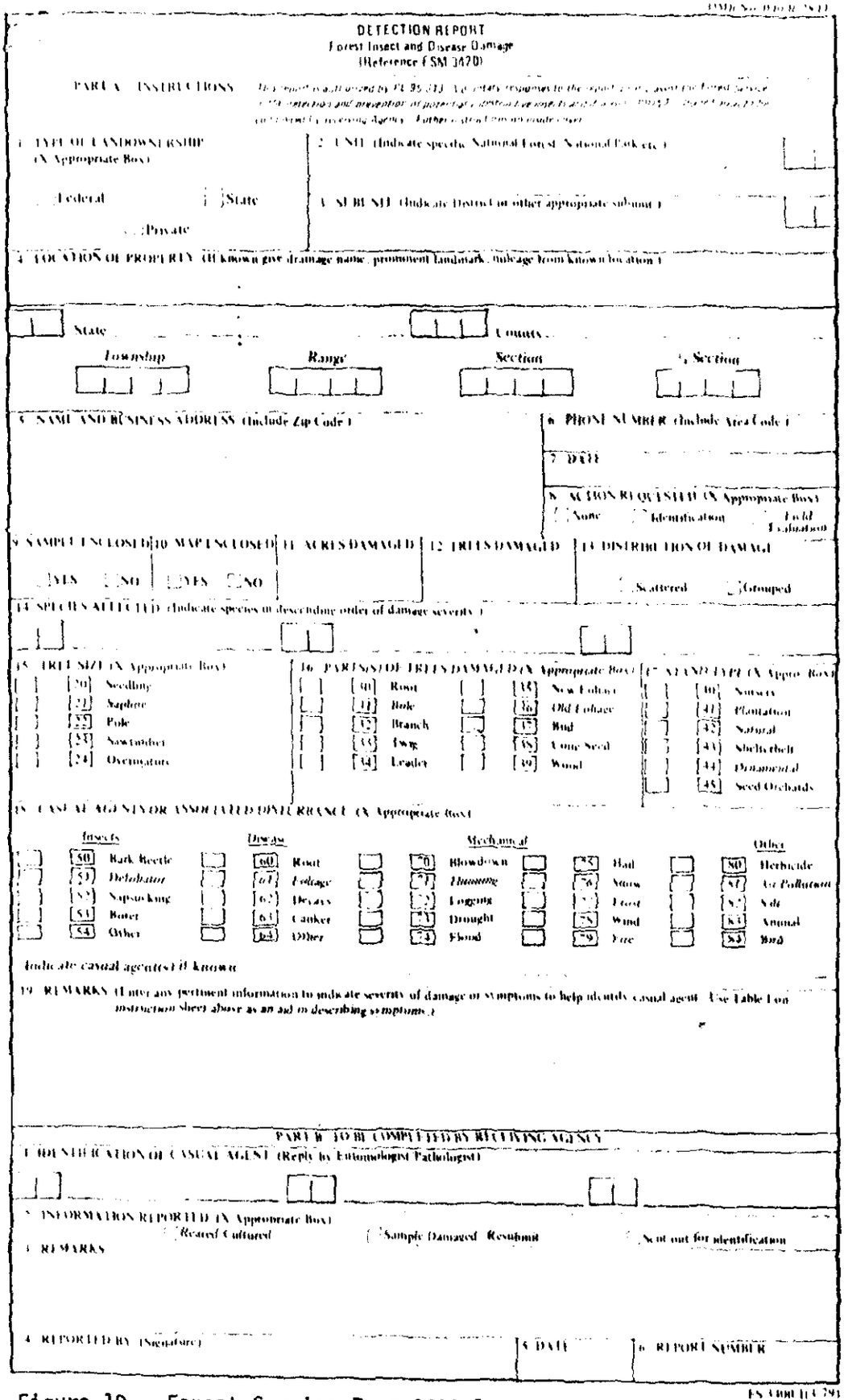
Oman, P. W. 1967. Collection and preservation of insects. USDA Misc. Pub. No. 601.

Peace, T. R. 1962. Pathology of trees and shrubs with special reference to Britain. London: Oxford Univ. Press. P. 12-13.

Pirone, P. P. 1972. Tree maintenance. New York, Oxford Univ. Press.

Sabrosky, Curtis W. 1971. Packing and shipping of pinned insects. Bull. Entomol. Soc. Amer. 17:6-8.

Shurtleff, Malcolm C. 1966. How to control plant diseases. Amer, Iowa State Univ. Press.



DETECTION REPORT
Forest Insect and Disease Damage
(Reference FSM 3420)

PART A - INSTRUCTIONS

1 TYPE OF LANDOWNERSHIP (X Appropriate Box)
( ) Federal ( ) State ( ) Private

2 UNIT (Indicate specific National Forest, National Park, etc.)

3 SUBUNIT (Indicate District or other appropriate subunit.)

4 LOCATION OF PROPERTY (If known give drainage name, prominent landmark, mileage from known location.)

State ... County ...

Township ... Range ... Section ... ¼ Section ...

5 NAME AND BUSINESS ADDRESS (Include Zip Code.)

6 PHONE NUMBER (Include Area Code.)

7 DATE

8 ACTION REQUESTED (X Appropriate Box)
( ) None ( ) Identification ( ) Field Evaluation

9 SAMPLE ENCLOSED ( ) YES ( ) NO

10 MAP ENCLOSED ( ) YES ( ) NO

11 ACRES DAMAGED

12 TREES DAMAGED

13 DISTRIBUTION OF DAMAGE ( ) Scattered ( ) Grouped

14 SPECIES AFFECTED (Indicate species in descending order of damage severity.)

15 TREE SIZE (X Appropriate Box)
[ ] [30] Seedling
[ ] [31] Sapling
[ ] [32] Pole
[ ] [33] Sawtimber
[ ] [34] Overmature

16 PARTS/SIDE TREES DAMAGED (X Appropriate Box)

[ ]	[40] Root	[ ]	[38] New Foliage	[ ]	[00] Nursery
[ ]	[41] Bole	[ ]	[36] Old Foliage	[ ]	[41] Plantation
[ ]	[42] Branch	[ ]	[37] Bud	[ ]	[42] Natural
[ ]	[43] Twig	[ ]	[38] Cone Seed	[ ]	[43] Shelterbelt
[ ]	[44] Leader	[ ]	[39] Wood	[ ]	[44] Ornamental
				[ ]	[45] Seed Orchards

17 STAND TYPE (X Appro. Box)

18 CASUAL AGENTS OR ASSOCIATED DISTURBANCE (X Appropriate Box)

Insects		Diseases		Mechanical			Other	
[ ] [50]	Bark Beetle	[ ] [60]	Root	[ ] [70]	Blowdown	[ ] [78]	Hail	[ ] [80]	Herbicide
[ ] [51]	*Defoliator*	[ ] [61]	*Foliage*	[ ] [71]	*Thinning*	[ ] [76]	Snow	[ ] [81]	*Air Pollutant*
[ ] [52]	Sapsucking	[ ] [62]	Decays	[ ] [72]	Logging	[ ] [77]	Frost	[ ] [82]	Salt
[ ] [53]	Borer	[ ] [63]	Canker	[ ] [73]	Drought	[ ] [78]	Wind	[ ] [83]	Animal
[ ] [54]	Other	[ ] [64]	Other	[ ] [74]	Flood	[ ] [79]	Fire	[ ] [84]	Bird

Indicate casual agent(s) if known

19 REMARKS (Enter any pertinent information to indicate severity of damage or symptoms to help identify casual agent. Use Table I on instruction sheet above as an aid in describing symptoms.)

PART B - TO BE COMPLETED BY RECEIVING AGENCY

1 IDENTIFICATION OF CASUAL AGENT (Reply by Entomologist/Pathologist)

2 INFORMATION REPORTED (X Appropriate Box)
( ) Received/Cultured ( ) Sample Damaged - Resubmit ( ) Sent out for identification

3 REMARKS

4 REPORTED BY (Signature) ... 5 DATE ... 6 REPORT NUMBER

FS-3400-1 (4-79)

Figure 10. Forest Service Form 3400-1